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| Institutional Animal Care and Use Committee | | UNTHSC |
| Title: The Use of Urethane in Rodents | | |
| Document #: 058 | Version #: 01 | |
| Approved by IACUC Date: January 28, 2020 | | |

A. BACKGROUND INFORMATION

- a. Urethane (ethyl carbamate) is a long-acting anesthetic used in laboratory animals during procedures of exceptionally long duration, in particular for recording procedures where the preservation of neural transmission and autonomic reflexes are essential.
- b. The purpose of this document is to describe the recommended methods for the use of urethane, as it has been known to cause adverse post-operative health effects in animals, and contain carcinogenic and mutagenic properties. It also poses a health risk to laboratory personnel, specifically pregnant women due to the antimetabolic potential of this compound.
- c. This document provides guidance regarding the safety for personnel in the preparation, usage, storage and proper disposal when using urethane.
- d. Urethane must be approved in the IACUC protocol with appropriate justification prior to use in laboratory animals.

B. RESPONSIBILITIES

- a. It is the responsibility for the Principal Investigator (PI) at the University of North Texas Health Science Center to follow the procedures set forth below for using urethane.
- b. It is the responsibility for the PI to appropriately train research personnel for the safe use of this anesthetic/ euthanasia agent in the species approved on the protocol.

C. PROCEDURES

- a. Formulation
 - i. The IACUC strongly recommends using pharmaceutical grade anesthetics; however, if the need arises for using urethane, justification for the use of a non-pharmaceutical grade compound must be provided in the IACUC protocol, in accordance to SOP 011: Use of Non-Pharmaceutical Grade Compounds in Animals.
- b. Safety Precautions
 - i. Personnel using urethane should be familiar with the SDS, which should be readily available in the laboratory. Questions regarding safety practices should be directed to UNTHSC Environmental Health and Safety.
 - ii. Urethane is considered carcinogenic, and may form combustible dust. Urethane solution can be absorbed through the skin. Safety precautions should be employed when used.
 - iii. Accidental ingestion or exposure seek medical attention if cough or other symptoms appear, such as discomfort, irritation, or vomiting. Provide SDS documentation when seeking medical attention.
 - iv. To prevent exposure, appropriate PPE should be worn, including gloves, lab coat, and eye protection. In order to prevent inhaling the volatilized drug, preparation should be conducted inside a certified fume hood and solution should be stored in a tightly sealed container.
- c. Preparation

- i. Urethane comes in a powdered or crystalized form, and must be stored in a tightly closed container in a cool, dry area. Because solid urethane is hygroscopic, open time for bottles should be kept to a minimum and purchase in large quantities is not recommended. Urethane may only be used by personnel who have been trained in the safe handling of this hazardous chemical. Other laboratory members must be informed when urethane is in use and avoided altogether when pregnant.
 - ii. Urethane should be kept away from incompatible materials, such as ignition sources, oxidizers, and sunlight.
 - iii. Urethane solutions must be prepared in a certified fume hood. Urethane may be prepared in sterile, non-pyrogenic water. Other solutions should be avoided, given the hypertonicity of the urethane solution already.
 - iv. Containers of urethane solution must be **labeled** with the agent and expiration date, concentration, and date of preparation, along with the initials of the individual who prepared the solution. Expiration date of the mixed solution is six months from the date in which the solution is prepared, or the expiration date of the stock drug or diluent used to make the solution, whichever date is first.
- d. Use in Rodents: Terminal Procedure
- i. Dose: Recommended urethane dose for rats is 1.3-1.5 g/kg in a ~1.5 g/5 ml solution. This dose does not need to be supplemented if animal is initially well-anesthetized. Anesthesia may last up to 24 hours and 1.5 g/kg is lethal (Field et.al, 1993).
 - ii. Route of Administration: Urethane should be given intraperitoneal (IP) for use with euthanasia, and intravenously (IV) for use in physiological experiments with rat initially anesthetized with a volatile anesthetic such as isoflurane. Giving urethane IP provides a fast, deep anesthesia ideal for euthanasia, but this route is osmotoxic to the mesenteric vasculature, causes peritoneal accumulation of fluid, and poor renal function. In contrast, IV administration of urethanes provides a stable, surgical plane of anesthesia (Severs et.al., 1981). Because a bolus dose of urethane IV is immediately lethal, urethane should be infused slowly, preferably by an infusion pump (~50 microliters/ min) as a volatile anesthesia such as isoflurane is withdrawn.
 - iii. Verification of anesthesia: Urethane produces loss of muscle tone and palpebral reflex (blink to light touch medial and lateral canthus of eye), and abolition of visible response to pinch of toe, tail, or abdomen for hours (Field et al., 1993), which must be verified to establish onset of adequate anesthesia and before administration of a paralytic. Test must be performed before administering supplements of paralytic agents.
 - iv. Blood Samples: Blood samples used for assays must be taken before administration of urethane to avoid contact and contamination. Tissues may not be shared with other labs.
- e. Disposal
- i. Empty containers should be relinquished as hazardous waste.
 - ii. Product or containers must not be disposed with household garbage.
 - iii. Please contact Environmental Health & Safety for proper disposal.

D. REFERENCES

- a. SOP 011: Use of Non-Pharmaceutical Grade Compounds in Animals
- b. OLAW Frequently Asked Questions – PHS Policy on Humane Care and Use of Laboratory Animals, F. Animal Use and Management, 4. May Investigators use Non-pharmaceutical grade substances in animals?
- c. Fisher Scientific, “Urethane,” 29CFR1910/1200 and GHS Rev.3, Oct 2014
- d. Field KJ and Lang CM. Hazards of urethane (ethyl carbamate): a review of the literature. *Laboratory Animals* 22: 255-262, 1988.
- e. Field KJ, White WJ, Lang CM. Anesthetic effects of chloral hydrate, pentobarbitone, and urethane in adult male rats. *Laboratory Animals* 27: 258-269, 1993.
- f. Severs WB, Keil LC, Klase PA, Deen KC. Urethane anesthesia in rats: Altered ability to regulate hydration. *Pharmacology* 22: 209-226, 1981.